STRUCTURE OF SUGAR-BEET PECTIN

Z. F. Ismailov, D. A. Rakhimov,
Z. R. Nogoibaeva, and N. P. Shelukhina

UDC 547.917

The results are given of a chemical study of one of the fractions of ammonium pectate, PAP-12 with a molecular weight of 18,000, obtained from sugar-beet pulp. PAP-12 contains mainly D-galacturonic acid residues in the pyranose form linked by α-1→4 bonds, and also rhamnose, galactose, and traces of arabinose.

A preparation of ammonium pectate has been isolated previously from sugar-beet pulp [1, 2]. In the present paper we give the results of a study of one of the fractions of the ammonium pectate – PAP-12. It consists of a grayish-white water-soluble powder, $[\alpha]_D^{20}$ +218 ±10° (c 1.0; water), which is precipitated completely by Cetavlon and by aluminum sulfate. PAP-12 contains 85.6% of uronic anhydride, determined by a method described elsewhere [3], and 1.38% of methoxy groups.

The complete acid hydrolysis of the substance formed mainly D-galacturonic acid, and also galactose and rhamnose in a ratio of 1:1.6, and traces of arabinose. The monosaccharides were identified by PC and GLC in comparison with authentic samples. The galacturonic acid was identified additionally by paper electrophoresis.

The preparation was homogeneous according to the results of ultracentrifugation and gel filtration. The molecular weight of the PAP-12 calculated from the results of ultracentrifugation was 18,000. Gel filtration on a column containing Sephadex G-100 calibrated with standard dextrans having molecular weights of 15,000, 20,000, and 40,000 gave a value of 17,900, agreeing with the results obtained from the rate of sedimentation [4]. The curve of the gel filtration of the polysaccharide showed a single peak with an elution volume V_e = 45 ml. The IR spectrum of PAP-12, like the spectra of pectins from other sources [5], had the following absorption bands (cm^{-1}): 1745 (stretching vibrations of the carbonyls of carboxy groups), 1600-1400 (vibration of an ionized carboxyl), 1000-1150 (stretching vibrations of a pyranose ring), and 760-960 (symmetrical and asymmetrical vibrations of a pyranose ring).

PAP-12 decomposed under the action of pectinase. Galacturonic acid was found in the products of enzymatic hydrolysis. It was found that the enzymatic hydrolyses of sugar-beet pectin and of apple pectin take place similarly. This permits the assumption of the presence of α-1 → 4 bonds between the galacturonic acid residues. The high value of the specific rotation of the pectin also indicates the α-configuration of the glycosidic bonds.

When PAP-12 was oxidized with sodium periodate, its consumption amounted to 0.63 mole per anhydro unit. The polyaldehyde obtained in this process, unlike the initial polysaccharide, rotated to the left, $[\alpha]_D^{20}$ −52° (c 2.0; water), reduced Fehling's solution, and gave a positive reaction with aniline phthalate. Hydrolysis of the polyalcohol obtained after the reduction of the polyaldehyde with sodium tetrahydroborate formed rhamnose and traces of galacturonic acid. The detection of rhamnose in this case shows that it is either a center of branching or is substituted at the C-3 hydroxyl.

By the partial hydrolysis of the PAP-12, a polysaccharide was isolated which, like the initial substance, had a high specific dextrorotation and consisted solely of D-galacturonic acid residues.

On the basis of the results of acid, enzymatic, and partial hydrolyses, IR spectroscopy, and periodate oxidation it has been established that PAP-12 consists mainly of galacturonic acid residues in the pyranose form connected by α-1 → 4 bonds, together with rhamnose, galactose, and traces of arabinose.

The pectin of sugar-beet pulp is similar to the pectin of sugar-beet leaves [6] but differs by a predominance of rhamnose among the neutral sugars.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Institute of Organic Chemistry, Academy of Sciences of the Kirghiz SSR, Frunze. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 35-38, January-February, 1980. Original article submitted September 29, 1979.

EXPERIMENTAL

The experiments were carried out with the sugar-beet pectin of the Nal'chik sugar refinery. The ammonium pectate was isolated by the method of Bhattecherjee and Timell [7].

The descending paper chromatography of sugars was carried out in the butan-1-ol–pyridine–water (6 : 4 : 3) system on FN-7 paper (GDR). The monosaccharides were detected with aniline hydrogen phthalate in water-saturated butanol at 110°C. For analysis, the monosaccharides were converted into the corresponding aldononitrile acetates [8], GLC being performed on a Tsvet-101 instrument with a flame-ionization detector using a steel column (200 × 0.3 cm). The liquid phase used was 5% of XE-60 on Chromaton N-AW (0.200-0.250 mm), at a rate of flow of helium of 60 ml/min and a temperature of the thermostat of 210°C and of the evaporator of 270°C. The relative amounts of sugars were determined from the areas of the peaks.

Paper electrophoresis was carried out in a horizontal instrument at 1100 V/7 mA in 1% acetic acid on FN-7 paper for 3 h. The IR spectra were recorded on a UR-20 instrument (tablets with KBr).

The polysaccharides were hydrolyzed in a sealed tube with 2 N H_2SO_4 (95-100°C, 40 h). All solutions were evaporated in vacuum at 40 ± 2°C. Ultracentrifugation was carried out on a MOM-3170 instrument at 30,000 rpm and a temperature of 20°C with an exposure interval of 5 min at an angle of rotation of 45°, the first exposure being made after 17 min. The concentration of the substance in aqueous solution was 1%. Found: $S = 1.41 \cdot 10^{-13}$, $D = 6.96 \cdot 10^{-7}$, mol. wt. 18,000.

For gel filtration we used Sephadex G-100 (fine) with 0.3% NaCl as eluent and solvent. The working volume of the column was 40 × 2 cm and the rate of flow 8 ml/h. The free volume of the column was determined with respect to dextran blue (V_0 = 25 ml). Calibration was carried out with dextrans having molecular weights of 15,000 (V_e = 50 ml), 20,000 (V_e = 40 ml), and 40,000 (V_e = 32 ml). On the column was deposited 1 ml of a 1% solution of the substance. The eluates were analyzed by the phenol/sulfuric acid method [9]. The molecular weight of the substance was determined from a calibration graph expressing the dependence of the elution volume V_e on the logarithm of $\overline{M}_n$ [10].

Enzymatic Hydrolysis. To a solution of 100 mg of the substance in 10 ml of water was added 10 mg of pectinase (Fluka) diluted with hydrochloric acid, pH 4. The mixture was incubated at 37°C for 48 h and was then boiled for 5 min to stop the enzymatic hydrolysis. In parallel, as control, apple pectin was hydrolyzed by the pectinase. The hydrolysis products were analyzed by the PC method, and galacturonic acid was detected.

Periodate Oxidation. The PAP-12 (110 mg) was oxidized with 0.05 M solution of sodium metaperiodate at room temperature in the dark. The consumption of periodate was 0.63 mole per anhydro unit. From the oxidation products was isolated a polyaldehyde with a yield of 0.09 g, $[\alpha]_D^{20}$ −52° (c 2.0; water). It gave a coloration with aniline phthalate and reduced Fehling's solution.

Smith Degradation. A solution of 80 mg of the polyaldehyde in 5 ml of water was treated with 0.2 g of $NaBH_4$ and the mixture was left at room temperature for 18 h. Then it was treated with Amberlite IR-120 (H^+) and was evaporated several times with methanol to eliminate boric acid. Rhamnose and traces of galacturonic acid were found in the hydrolysate by PC and GLC.

Isolation of the Galacturonan. The PAP-12 (0.3 g) was treated with 2 N sulfuric acid (1 : 30) at 100°C for 4 h. The precipitate of galacturonan that deposited was separated off and was washed with 1% sulfuric acid, 70% ethanol, acetone, and ether. Yield 0.145 g, $[\alpha]_D^{20}$ +275° (C 1.0; water). Galacturonic acid was found by PC and electrophoresis in the products of its complete acid hydrolysis. When the galacturonan was treated with pectinase as described above, galacturonic acid was formed.

SUMMARY

It has been established that the pectin fraction PAP-12 with a molecular weight of 18,000 from sugar-beet pulp consists mainly of D-galacturonic acid residues in the pyranose form linked by α-1 → 4 bonds, together with rhamnose, galactose, and traces of arabinose.

LITERATURE CITED

1. N. P. Shelukhina, G. B. Aimukhamedova, and Z. R. Nogoibaeva, Izv. Akad. Nauk Kirg. SSR, No. 3, 61 (1972).
2. Z. R. Nogoibaeva, N. P. Shelukhina, and G. B. Aimukhamedova, Izv. Akad. Nauk Kirg. SSR, No. 6, 58 (1976).

3. N. P. Shelukhina, Z. P. Nogoibaeva, and G. B. Aimukhamedova, An Investigation of the Fractional Composition of Sugar-Beet Pectin [in Russian], Frunze (1979).
4. T. J. Bowen, An Introduction to Ultracentrifugation, Wiley-Interscience, New York (1970).
5. M. P. Filippov, in: Infrared Spectra of Pectin Substances [in Russian], Kishinev (1973), p. 14.
6. M. S. Dudkin, L. V. Kaprel'yants, and V. E. Starichkova, Khim. Prir. Soedin., 321 (1975).
7. S. S. Bhattecherjee and T. E. Timell, Can. J. Chem., 43, 758 (1965).
8. D. G. Lance and J. K. N. Jones, Can. J. Chem., 45, 1965 (1967).
9. M. Dubois, K. L. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Anal. Biochem., 28, 350 (1956).
10. M. Tomoda and M. Uno, Chem. Pharm. Bull., 19, 1214 (1971).

DADI SPECTRA OF GOSSYPOL HEXAMETHYL ETHERS AND MIXTURES OF THEM

M. S. Abdugabbarov, N. I. Baram,
A. I. Ismailov, and R. Razakov

UDC 543.51:547.565.6

The DADI spectra of the M^+ ions of hexamethyl ethers of three tautomeric forms of gossypol and of mixtures of them have been studied. A specific fragmentation pathway of M^+ for each form has been shown. On the basis of the results obtained, the amounts of the hexamethyl ethers of each tautomeric form produced in the methylation of gossypol have been found.

The existence of gossypol – a natural aldehydonaphthol of plants of the family Malvaceae – in three tautomeric forms (aldehydic, lactol, quinoid) have been suggested by Adams, the existence of each of the forms being confirmed by the preparation of corresponding derivatives [1].

It was later shown by PMR spectroscopy that in weakly polar solvents gossypol exists predominantly in the dialdehyde form, while in aprotic solvents such as dimethyl sulfoxide a dynamic equilibrium is observed between the lactol and aldehyde forms [2].

The existence of tautomeric forms of gossypol can be followed most clearly by its methylation under various conditions [3, 4]. Mixtures of the tautomeric forms of gossypol hexamethyl ethers (GHMEs) are obtained from which it is possible to isolate only the dialdehyde form (I) by column chromatography on alumina. Various methods of methylating gossypol by means of various methylating agents are used to isolate the dilactol (II) and monolactol-monoaldehyde (III) forms. Consequently, the true quantitative ratio of the GHMEs formed and, therefore, the ratio of the tautomeric forms of gossypol preceding methylation has not hitherto been established.

Structural features of the GHMEs have been studied with the use of various spectrometric methods, including low-resolution mass spectrometry. In the last case, the peak of the molecular ion appears at maximum intensity, which shows the great stability of the GHME molecules to electron impact [4, 5].

The present paper gives the results of a study of the various tautomeric forms of the GHMEs in the individual state and of mixtures of them by the direct analysis of daughter ions (DADI) [6, 7].

The DADI spectra of the M^+ ions of the GHMEs differ greatly from one another, giving a kind of "fingerprint" of each of the isomers (Fig. 1, a–c).

Scheme 1 shows the possible structures of the fragmentary ions detected in the DADI spectra of the M^+ ions (the vertical dashed lines denote that the second half of the GHME molecule remains unchanged).

Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 38–42, January-February, 1980. Original article submitted August 28, 1979.